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(54) Antifungal compositions.

(57) Antifungal compositions of aculeacin A and specific fatty acids, such as oleic, linoleic, linolenic, lauric, palmitoleic, arachidonic and palmitelaidic surprisingly increase the intrinsic activity of aculeacin A against fungi, such as *Candida albicans*.

This is a continuation-in-part of application Serial No. 346,798, filed on February 8, 1982.

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- 1 -

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ANTIFUNGAL COMPOSITIONS

The instant invention relates to antifungal compositions containing aculeacin A and an amount of fatty acid selected from the group consisting of oleic acid, linoleic acid, linolenic acid, lauric acid, palmitoleic acid, arachidonic acid, and palmitelaidic acid sufficient to potentiate the bioactivity of aculeacin A. These compositions surprisingly increase the intrinsic activity of aculeacin A against fungi, such as Candida albicans strains B 311, BC 759 and B 3153A. These strains are maintained in the Smith Kline & French Laboratories culture collection.

Illustrative of the instant invention are antifungal compositions wherein between 5 and 3500 units by weight of oleic acid are combined with one unit of aculeacin A, provided that the minimum amount of aculeacin A is 0.039 μ g. Particular antifungal compositions are those where between 10 and 350 units by weight of oleic acid and one unit of aculeacin A are combined. Specific antifungal compositions of the instant invention contain between 50 and 3500 units by weight of oleic acid for each unit of aculeacin A, when at least 0.4 μ g of aculeacin A is employed.

Similarly, antifungal compositions of the instant invention contain between 5 and 360 units by weight of linoleic acid for each unit of aculeacin A, provided that the minimum amount of aculeacin A is 0.4 μ g. Particularly, antifungal compositions containing between 40 and 360

0086092

1 units by weight of linoleic acid and one unit of aculeacin
A are described when at least 0.625 ug of aculeacin A is
employed.

5 Further, antifungal compositions of the instant
invention contain between 5 and 180 units by weight of
linolenic acid and one unit of aculeacin A, provided that
the minimum amount of aculeacin A is 0.4 ug. Specifically,
between 40 and 180 units by weight of linolenic acid
and one unit of aculeacin A are combined to afford an
10 antifungal composition when at least 0.625 ug of aculeacin
A is employed.

Additional illustrations of the antifungal
compositions of the instant invention are those
compositions wherein between 250 and 1000 units by weight
15 of arachidonic acid and one unit of aculeacin A are
combined and wherein between 500 and 2000 units by weight
of palmitelaidic acid and one unit of aculeacin A are
combined when at least 1.0 ug of aculeacin is employed.

Aculeacin A is a known antifungal antibiotic
20 disclosed and described in the Journal of Antibiotics
30(4), pp. 297-313 (1977).

The antifungal activity of aculeacin A and the
compositions of aculeacin A and fatty acids was measured
by disc diffusion. Potentiation of antifungal activity
25 was examined by three different methods employing: (1) a
single concentration of aculeacin A and a single
concentration of known fatty acids; (2) various
concentrations of aculeacin A with a single concentration
of individual fatty acids; and (3) various concentrations
30 of fatty acids with a single concentration of aculeacin
A. The antifungal activities of aculeacin A and the fatty
acids were separately checked as controls. The fatty
acids, per se, employed in the compositions of this
invention do not exhibit antifungal activity at the
35 concentrations tested.

The effects of a number of fatty acids on the
antifungal activity of aculeacin A were determined by

1 utilizing seeded plates of Candida albicans B 311 in yeast
nitrogen base (YNB) agar (Difco) with glucose as the
carbon source. A composition containing one unit by
weight of aculeacin A and 5 units of fatty acid or the
5 sodium salt thereof was spotted on the seeded plates and
the size of the zone of inhibition after 16-18 hours
incubation at 28-37°C was measured. The results of the
above test are shown in Table I.

10

TABLE I

	<u>Fatty acid</u>	<u>Zone Size (mm)</u>
	none (control)	15
	Oleic Acid (sodium salt)	32
	Palmitoleic Acid	26
15	Lauric Acid	22
	Linoleic Acid	23
	Linolenic Acid	22
	Stearic Acid	15
	Palmitic Acid	15
20	Myristic Acid	16
	Decanoic Acid	17
	Caprylic Acid	16

25 Aculeacin A and fatty acid compositions of Table
I which afford an increase of 3 millimeters over aculeacin
A demonstrated sufficient increase in the intrinsic
activity of aculeacin A to be deemed synergistic.

30 Additional fatty acids were tested to determine
if they increased the intrinsic activity of aculeacin A
against C. albicans B 311 utilizing seeded plates of YNB
agar (Difco) with lysine as the carbon source and seeded
plates of Sabouarud dextrose agar (Difco). Compositions
containing one µg of aculeacin A and various amounts of the
fatty acid was spotted on the seeded plates and the size
35 of the zone of inhibition after 16-18 hours incubation at
37°C was measured. The results of the above tests are
shown, as well as a control, are shown in Table II.

0086092

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TABLE II

	<u>Aculeacin A (ug)</u>	<u>Fatty Acid (ug)</u>	<u>Zone Size (mm)</u>	
			<u>YNB agar</u>	<u>Sabouarud Agar</u>
	1	None (control)	15	13
5	1	None (control)	16	15
<u>Erucic Acid</u>				
	0 (control)	1000	0	0
	1	1000	15	Trace
10	0 (control)	500	0	0
	1	500	17	17
	1	500	15	17
	0 (control)	250	0	0
<u>Vaccinic Acid</u>				
15	0 (control)	2000	0	0
	1	2000	17	26
	0 (control)	1000	0	0
	1	1000	17	25
20	0 (control)	500	0	0
	1	500	17	25
<u>Linoleic Acid</u>				
	0 (control)	1000	0	0
25	1	1000	16	24*
	0 (control)	500	0	0
	1	500	17	20*
	0 (control)	250	0	0
	1	250	26	20*
30	<u>Arachidonic Acid</u>			
	0 (control)	1000	0	0
	1	1000	26	20*
	0 (control)	500	0	0
35	1	500	20	20*
	0 (control)	250	0	0
	1	250	20	20*

0086092

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TABLE II (continued)

Homo- γ -Linoleic Acid

	0 (control)	200	0	0
5	1	200	17*	15
	0 (control)	100	0	0
	1	100	15*	15
	0 (control)	50	0	0
	1	50	15*	15

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Palmitelaidic Acid

	0 (control)	2000	0	0
	1	2000	22	25
15	0 (control)	1000	0	0
	1	1000	20	32
	0 (control)	500	0	0
	1	500	20	30

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α -Hydroxy Lauric Acid

	0 (control)	1000	Trace	10 (hazy)
	1	1000	25	20
	0 (control)	500	Trace	10 (hazy)
	1	500	24	15
25	0 (control)	250	Trace	Trace
	1 (control)	250	20	15

γ -Linoleic Acid

	0 (control)	2000	0	0
30	1	2000	22	25
	0 (control)	1000	0	0
	1	1000	15	24
	0 (control)	500	0	0
35	1	500	14	20

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TABLE II (continued)

Behenic Acid

	0 (control)	1000	0	0
	1	1000	15	20
5	0 (control)	500	0	0
	1	500	14	14
	0 (control)	250	0	0
	1	250	14	15

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Eladic Acid

	0 (control)	1000	0	0
	1	1000	13	13
	0 (control)	500	0	0
15	1	500	13	15
	0 (control)	250	0	0
	1	250	13	18

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Oleic Acid
Sodium Salt

	0 (control)	1000	0	0
	1	1000	35	26
	0 (control)	500	0	0
	1	500	35	26
25	0 (control)	250	0	0
	1	250	35	28

*irregular zone of inhibition

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Aculeacin A and fatty acid compositions of Table II which afforded an increase of 3 millimeters over Aculeacin A in both the YNB agar and the Sabouarud dextrose agar demonstrated a sufficient increase in the intrinsic activity of aculeacin A to be deemed synergistic. In addition to the above described compositions, the composition of 250 units by weight linoelaidic acid to one unit aculeacin A and the

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0086092

1 composition of 2000 units by weight γ -linoleic acid to one
unit aculeacin A exhibit a surprising increase in the
intrinsic activity of aculeacin A.

5 The antifungal effects of compositions containing
a single amount of oleic acid as the sodium salt while
varying the amounts of aculeacin A were determined by
utilizing seeded plates of Candida albicans B 311 in
Sabouraud dextrose agar and measuring the zone of
inhibition after 16-18 hours incubation at 28-37°C. The
10 results of the above test, as well as a control, are shown
in Table III.

TABLE III

	<u>Aculeacin A (ug)</u>	<u>Oleic Acid (ug)</u>	<u>Zone Size (mm)</u>
15	2.5	0	19
	1.25	0	14-16
	1.25	12.5	37
	0.625	0	10
	0.625	12.5	35
20	0.312	0	trace
	0.312	12.5	35
	0.156	12.5	32
	0.078	12.5	29
	0.039	12.5	28
25	0.020	12.5	trace
	0.000	12.5	0

The antifungal effects of compositions containing
a single amount of aculeacin A while varying the amounts .
30 of oleic acid as the sodium salt were determined by
utilizing seeded plates of Candida albicans strains B 311,
BC 759 and B 3153A and measuring the zone of inhibition
after 16-18 hours at 28-37°C. This test was also
performed with linoleic acid and linolenic acid. The
35 results of these tests are shown in Table IV-a, IV-b and
IV-c.

TABLE IV-a					
1	<u>Aculeacin A (ug)</u>	<u>Oleic Acid (ug)</u>	<u>Zone Size (mm)</u>		
			<u>B 311</u>	<u>BC 759</u>	<u>B 3153-A</u>
	0.40	0	10	13	12
5	0.40	5	13	13	13
	0.40	10	14	15	15
	0.40	20	15	18	18
	0.40	40	17	18	17
	0.40	78	20	20	18
10	0.40	156	26	24	25
	0.40	312	30	28	25
	0.40	625	32	29	28
	0.40	1250	35	32	29

		TABLE IV-b	
15	<u>Aculeacin A (ug)</u>	<u>Linoleic Acid (ug)</u>	<u>Zone Size (mm)</u>
			<u>B 311</u>
	0.625	225	19
	0	225	Trace
		112.5	21
20	0.625	112.5	0
	0	56.2	22
	0.625	56.2	0
	0	28.1	22
	0.625	28.1	0
25	0	0	16
	0.625		

		TABLE IV-c	
30	<u>Aculeacin A (ug)</u>	<u>Linolenic Acid (ug)</u>	<u>Zone Size (mm)</u>
			<u>B 311</u>
	0.625	112.5	19
	0	112.5	0
	0.625	56.2	19
	0	56.2	0
	0.625	28.1	20
35	0	28.1	0
	0.625	0	16

1 The seeded plates of Candida albicans strains
B 311, BC 759, and B 3153A were prepared by inoculating 50
ml of trypticase soy broth (BBL) with one ml of the
preserved frozen culture and incubating the inoculum at
5 37°C for 7 hours on a rotary shaker. Two milliliters of
this culture were used to inoculate one liter of Sabouraud
dextrose agar (Difco) at 50°C and 15 milliliters of the
resultant medium poured into 150 mm petri-dishes.

 The antifungal activity of the compositions of
10 the instant invention, as exemplified by the composition
containing Aculeacin A and oleic acid as shown below, was
demonstrated by topical treatment of C. albicans
infections of mice. Clinical isolates of C. albicans were
grown in Sabouraud dextrose agar and subcultured into
15 mouse serum and incubated at 35°C. Female mice (CF-1, 4
to 6 weeks old) were shaved on the back (2cm² area).
The shaved area was cleaned with 70% alcohol and scrubbed
with a wire brush. This area was then painted with the C.
albicans in mouse serum as noted above (10⁵-10⁶
20 CFU/ml.) and after drying this area was covered with
sterile gauze and the infection was allowed to develop
over two days. After the development of the infection,
the mice were treated with aculeacin A/oleic acid
composition, aculeacin A and Lotrimin^R (Clotrimazole-
25 Schering) for 5 days. The minimum inhibitory
concentration (MIC) for aculeacin A for each clinical
isolate of C. albicans was determined and that amount was
employed in each experiment. Commercially available
Lotrimin^R cream was utilized in each experiment. As the
30 results below demonstrate, the aculeacin A/oleic acid
composition afforded a significant reduction in the
infection (comparable to Lotrimin^R) over aculeacin A,
itself, or the control. Oleic acid was tested separately
and did not inhibit the infection. Note that bacterial
35 contamination was ruled out by microscopic examination.

Experiment Number	Untreated Control	Lotrimin ^R	Aculeacin A (ug)	Aculeacin A/Oleic Acid (ug/ug)
I	4 3	0 0	1 1 (0.8)	0 0 (0.5/100)
II	2 2	1 1	0 1 (1.5)	0 0 (0.9/100)
III	3 3	0 0	1 1 (5.0)	0 0 (1.5/100)
IV	3 3	0 0	2 2 (0.5)	1 0 (0.5/100)
V	3 3	0 0	0 0 (10.5)	0 0 (5.0/100)
VI	3 3	0 0	1 1 (5.0)	1 1 (5.0/100)

where 4 - the most severe infection - zone of inoculation highly erythrematous with culture mainly consisting of C. albicans.
 3 - severe infection with some healing
 2 - less severe infection
 1 - very little infection
 0 - no infection.

In view of the above described antifungal activity, antifungal amounts of the compositions of the instant invention may be employed as the active ingredients in pharmaceutically acceptable vehicles, such as solid, semi-solid or liquid carriers, to combat fungus growth. The instant compositions in pharmaceutical form may be applied to the area to be treated by conventional methods, such as, dusting, spraying, brushing, smearing, impregnating or other suitable means. The instant compositions may also be employed in agricultural forms to treat fungus growth in cultivated fields.

0086092

Claims:

1. A composition comprising an antifungal effective amount of aculeacin A and an amount of fatty acid selected from the group consisting of oleic acid, linoleic acid, linolenic acid, lauric acid, palmitoleic acid, arachidonic acid and palmitelaidic sufficient to potentiate the bioactivity of aculeacin A.

2. An antifungal composition of claim 1 which is effective against Candida albicans infection.

3. An antifungal composition of claim 1 wherein the fatty acid is oleic acid.

4. An antifungal composition of claim 3 wherein the amount of oleic acid is between 5 and 3500 units by weight for each unit of aculeacin A, provided that the minimum amount of aculeacin A is 0.039 μg .

5. An antifungal composition of claim 4 wherein the amount of oleic acid is between 10 and 350 units by weight for each unit of aculeacin A.

6. An antifungal composition of claim 4 wherein the amount of oleic acid is between 50 and 3500 units by weight for each unit of aculeacin A, when at least 0.4 μg of aculeacin A is employed.

7. An antifungal composition of claim 1 wherein the fatty acid is linoleic acid.

8. An antifungal composition of claim 7 wherein the amount of linoleic acid is between 5 and 360 units by weight for each unit of aculeacin A, provided that the minimum amount of aculeacin A is 0.4 μg .

9. An antifungal composition of claim 8 wherein the amount of linoleic acid is between 40 and 360 units by weight for each unit of aculeacin A when at least 0.625 μg of aculeacin A is employed.

10. An antifungal composition of claim 1 wherein the fatty acid is linolenic acid.

11. An antifungal composition of claim 10 wherein the amount of linolenic acid is between 5 and 180 units by weight for each unit of aculeacin A, provided that the minimum amount of aculeacin A is 0.4 μg .

12. An antifungal composition of claim 11 wherein the amount of linolenic acid is between 40 and 180 units by weight for each unit of aculeacin A when at least 0.625 µg of aculeacin A is employed.

5 13. An antifungal composition of claim 1 wherein the fatty acid is arachidonic acid.

14. An antifungal composition of claim 12 wherein the amount of arachidonic acid is between 250 and 1000 units by weight for each unit of aculeacin A when at least 1.0 µg of aculeacin A is employed.

15 15. An antifungal composition of claim 1 wherein the fatty acid is palmitelaidic acid.

16. An antifungal composition of claim 15 wherein the amount of palmitelaidic acid is between 500 and 2000 units by weight for each unit of aculeacin A when at least 1.0 µg of aculeacin is employed.

17. A composition comprising an antifungal effective amount of aculeacin A and an amount of a fatty acid selected from the group consisting of lineolaidic acid and γ-linoleic acid sufficient to potentiate the bioactivity of aculeacin A wherein the amount of lineolaidic acid is 250 units by weight to one unit of aculeacin A and wherein the amount of γ-linoleic acid is 2000 units by weight to one unit of aculeacin A.

- 13 -

Claim (Austria)

1. A process for the preparation of an anti-fungal composition comprising an antifungal effective amount of aculeacin A and an amount of fatty acid
5 selected from the group consisting of oleic acid, linoleic acid, linolenic acid, lauric acid, palmitoleic acid, arachidonic and palmitelaidic acid sufficient to potentiate the bioactivity of aculeacin A; which
10 comprises mixing the required amounts of aculeacin A and fatty acid.